

Vent Polymerase PCR

Reaction Set-up:

Material	Volume	Concentration
dH ₂ O	37 QS 50µL	-
ThermoPol Buffer	5 µL	-
dNTPs (50x)	1 µL	10 mM
Fwd Primer (20x)	2.5 µL	10 µM
Rev Primer (20x)	2.5 µL	10 µM
Template	Variable (~1uL)	2-10 ng
VentR DNA Polymerase (100x)	1 µL	100x stock

Thermal Cycling:

Temp. (°C)	Time	Cycle
95	5:00	
95	0:30	
*	0:30	25X
72	†	
72	10:00	
4	∞	

*Lowest primer annealing temperature

†1min per 1kb

Phusion Polymerase PCR

Material	Volume	Concentration
dH2O	32 QS 50µL	-
5x Phusion HF Buffer	10 µL	-
dNTPs (50x)	1 µL	10 mM
Fwd Primer (20x)	2.5 µL	10 µM
Rev Primer (20x)	2.5 µL	10 µM
Template	Variable (~1uL)	
Phusion DNA Polymerase (100x)	1 µL	100x stock

Thermocycling:

Temp. (°C)	Time	Cycle
98	0:30	
98	0:10	
45-72*	0:30	25-35X
72	0:30/kb	
72	10:00	
4	∞	

*Round down T_m for lowest annealing temperature primer

Colony PCR

Material	Volume	Concentration
dH2O	67 QS 100µL	-
ThermoPol Buffer	5 µL	-
dNTPs (50x)	1 µL	10 mM
Fwd Primer (20x)	2.5 µL	10 µM
Rev Primer (20x)	2.5 µL	10 µM
VentR DNA Polymerase (100x)	1 µL	100x stock

1. Suspend colonies in 20µL of dH2O using toothpick
2. Transfer toothpick to 3mL LB + Ab
3. Add 1µL of cell suspension to 9µL master mix
4. Thermal Cycling:

Temp. (°C)	Time	Cycle
95	0:30	
95	0:30	
*	0:30	35X
68	0:30†	
68	5:00	
4	∞	

*Lowest primer annealing temperature

†30sec per 1kb

5. Grow correct length colonies overnight
6. Spot 3µL onto master plate (for later recovery)
7. Miniprep remaining culture and send for sequencing
8. After successful sequencing:

Day 1:

1. Start 2 overnight liquid culture (16-18 hours) of correct colony from master plate

Electroporation

1. Make [S.O.C. Broth](#)
2. Thaw, on ice, XL1 EC cells (40µL)
3. Add DNA (1-3µL) to cells, flick to mix, let sit 1min on ice
4. Transfer cells to electroporation cuvette
5. Electroporate cells (~5ms)
6. Add 1mL [S.O.C. Broth](#), pipette to mix, transfer to culture tube

7. Incubate @ 37C shaking for 1hr
8. Plate 100µL of 1:100 and 1:1000 dilution onto appropriate plate
9. Save tube in 4C

S.O.C. Broth

Volume	Name	Concentration
1 mL	Super Optimal Broth	-
10 µL	MgSO ₄ (10x)	1000 mM
10 µL	MgCl ₂ (10X)	1000 mM
20 µL	Glucose (20X)	1000 mM